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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c)

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TITLE OF THE INVENTION (280 characters max)

SELECTIVE ESTROGEN RECEPTOR MODULATORS

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,
SIGNATURE G-T Voy

Date 6 25 / 04

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REGISTRATION NO. (*if appropriate*) 43,972

☐ Additional inventors are being named on separately numbered sheets attached hereto

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SELECTIVE ESTROGEN RECEPTOR MODULATORS

Field of Invention

The present invention is in the field of medicine, particularly in the treatment of gynecological disorders. More specifically, the present invention relates to selective estrogen receptor modulators useful to treat endometriosis and uterine fibrosis.

Background of the Invention

Uterine leiomyoma/leiomyomata (uterine fibroid disease) is an old and ever present clinical problem that goes under a variety of names, including uterine fibrosis, uterine hypertrophy, uterine lieomyomata, myometrial hypertrophy, fibrosis uteri, and fibrotic metritis. Essentially, uterine fibrosis is a condition where there is an inappropriate deposition of fibroid tissue on the wall of the uterus. This condition is a cause of dysmenorrhea and infertility in women.

Endometriosis is a condition of severe dysmenorrhea, which is accompanied by severe pain, bleeding into the endometrial masses or peritoneal cavity and often leads to infertility. The symptom's cause appears to be ectopic endometrial growths that respond inappropriately to normal hormonal control and are located in inappropriate tissues. Because of the inappropriate locations for endometrial growth, the tissue seems to initiate local inflammatory-like responses causing macrophage infiltration and a cascade of events leading to initiation of the painful response. Evidence suggests that a cause of uterine fibrosis and endometriosis is an inappropriate response of fibroid tissue and/or endometrial tissue to estrogen.

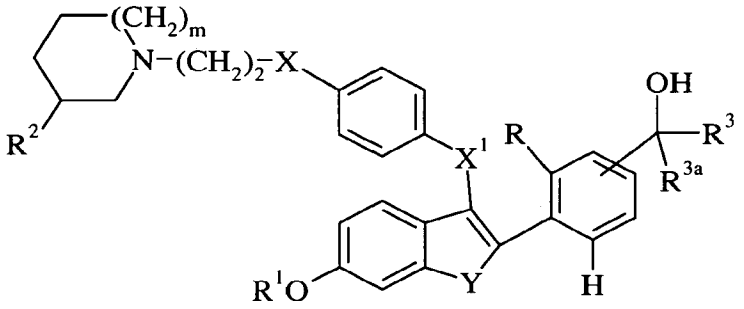
Many publications have appeared within the last ten years disclosing novel selective estrogen receptor modulators (SERMs), *e.g.*, U.S. Patent No.'s 5,484,795, 5,484,798, 5,510,358, 5,998,401 and WO 96/09040. Many of these SERMs, generally

speaking, have been found to have a beneficial estrogen agonist activity in the bone and cardiovascular systems with a concomitant beneficial estrogen antagonist activity in the breast. A small, particularly useful subset of such compounds has also been found to have an estrogen antagonist effect in the uterus. A compound with this particularly useful SERM profile holds particular promise in treating uterine leiomyoma/leiomyomata and/or endometriosis.

However, the actual use of these SERM compounds, particularly in pre-menopausal women, has been hampered due to said compound's stimulatory effect on the ovaries. A great need currently exists, therefore, for new SERM compounds that behave as estrogen antagonists in the uterus that do not stimulate the ovaries.

Summary of Invention

The present invention relates to a compound of formula I:



I;

wherein:

m is 0, 1 or 2;

R^1 is H, SO_2(n-C_4-C_6 alkyl) or COR^4;

R^2 is H or methyl provided that if m is 1 or 2, then R^2 must be H and that if m is 0, then R^2 must be methyl;

R^3 and R^{3a} are independently H or C_1-C_6 alkyl;

X is O or NR^5;

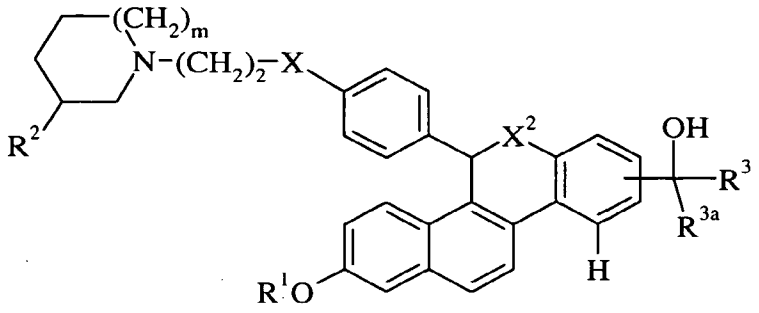
Y is S or CH=CH;

R^4 is C_1-C_6 alkyl, C_1-C_6 alkoxy, NR^6R^7, phenoxy, or phenyl optionally substituted with halo;

R^5 is H or C_1-C_6 alkyl;

R^6 and R^7 are independently H, C_1-C_6 alkyl or phenyl;

X^1 is O, CH_2 or CO and R is H or R combines with X^1 to form a moiety of the formula:



wherein m, R^1, R^2, R^3, R^{3a} and X are as defined above; and

X^2 is O or S;

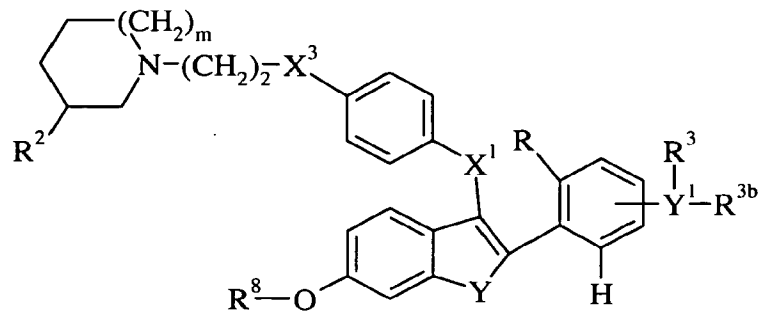
or a pharmaceutical acid addition salt thereof.

The present invention also relates to a pharmaceutical composition containing a compound of formula I and a pharmaceutical carrier. In another embodiment, the pharmaceutical composition of the present invention may be adapted for use in treating endometriosis and/or uterine fibrosis.

The present invention also relates to methods for treating endometriosis and/or uterine fibrosis employing a compound of formula I.

In addition, the present invention relates to a compound of formula I for use in treating endometriosis and/or uterine fibrosis. The present invention is further related to the use of a compound of formula I for the manufacture of a medicament for treating endometriosis and/or uterine fibrosis.

The present invention further relates to a compound of formula II:



II;

wherein:

m is 0, 1 or 2;

R^2 is H or methyl provided that if m is 1 or 2, then R^2 must be H and that if m is 0, then R^2 must be methyl;

R^3 is H or C_1-C_6 alkyl;

R^{3b} is absent or is H or C_1-C_6 alkyl provided that if Y^1 is C(OH), then R^{3b} is H or C_1-C_6 alkyl and that if Y^1 is C=O, then R^{3b} is absent;

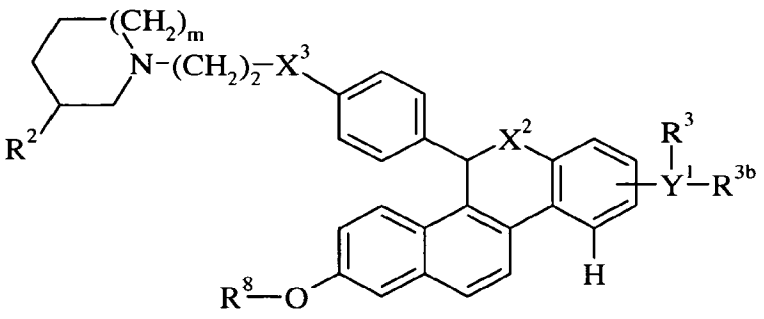
R^8 is H, C_1-C_6 alkyl, benzyl, SO_2CH_3, SO_2(n-C_4-C_6 alkyl) or COR^4;

X^3 is O or NR^9;

Y is S or CH=CH;

Y^1 is C=O or C(OH);

X^1 is O, CH_2 or CO and R is H or R combines with X^1 to form a moiety of the formula:



;

wherein m, R^2, R^3, R^{3b}, R^8, X^3 and Y^1 are as defined above; and

X^2 is O or S;

R^4 is C_1-C_6 alkyl, C_1-C_6 alkoxy, NR^6R^7, phenoxy, or phenyl optionally substituted with halo;

R^6 and R^7 are independently H, C_1-C_6 alkyl or phenyl;

R^9 is H, C_1-C_6 alkyl or $CO_2(C_1$-C_6 alkyl); and

provided that if Y^1 is C(OH), then R^8 is C_1-C_6 alkyl, SO_2CH_3 or benzyl or X^3 is NR^9 and R^9 is $CO_2(C_1$-C_6 alkyl); or an acid addition salt thereof; useful as an intermediate to a compound of formula I.

Detailed Description

Unless specified otherwise, reference hereafter to a "compound of formula I" includes the pharmaceutical acid addition salts thereof.

The compounds of the present invention have one or more chiral centers and may exist in a variety of stereoisomeric configurations. As a consequence of these chiral centers, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All such racemates, enantiomers, and diastereomers are within the scope of the present invention.

For the purposes of the present invention, as disclosed and claimed herein, the following terms are defined below.

The term "halo" refers to fluoro, chloro, bromo and iodo. The term "C_1-C_6 alkyl" represents a straight, branched or cyclic hydrocarbon moiety having from one to six carbon atoms, *e.g.*, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl and the like. Moieties such as a cyclobutylmethylene are also included within the scope of a C_1-C_6 alkyl group. The term "C_1-C_4 alkyl" refers specifically to methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl, t-butyl and cyclobutyl. The term "n- C_4-C_6 alkyl" refers specifically to n-butyl, n-pentyl and n-hexyl. A "C_1-C_6 alkoxy" group is a C_1-C_6 alkyl moiety connected through an oxy linkage.

The term "pharmaceutical" when used herein as an adjective means substantially non-deleterious.

A pharmaceutical "acid addition salt" is a salt formed by reaction of the free base form of a compound of formula I with a pharmaceutical acid, such as described in the Encyclopedia of Pharmaceutical Technology, editors James Swarbrick and James C. Boylan, Vol 13, 1996 "Preservation of Pharmaceutical Products to Salt Forms of Drugs and Absorption". Specific salt forms include, but are not limited to the: acetate, benzoate, benzenesulfonate, 4-chlorobenzenesulfonate; citrate; ethanesulfonate; fumarate; d-gluconate; d-glucuronate; glutarate; glycolate; hippurate; hydrochloride; 2-hydroxyethanesulfonate; dl-lactate; maleate; d-malate; l-malate; malonate; d-mandelate; l-mandelate; methanesulfonate; 1,5 napthalenedisulfonate; 2-naphthalenesulfonate; phosphate; salicylate; succinate; sulfate; d-tartrate; l-tartrate; and p-toluenesulfonate.

The term "patient" as used herein refers to female humans and non-human female animals such as companion animals (dogs, cats, horses and the like).

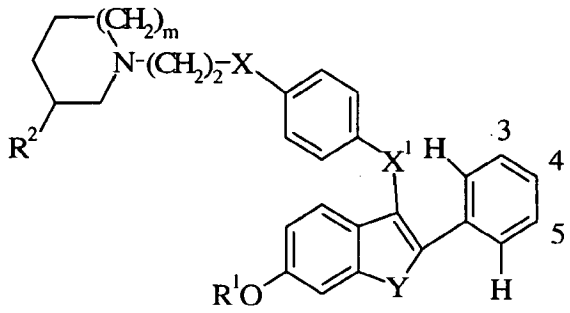
The terms "treating" and "treat" as used herein means alleviating, ameliorating, preventing, prohibiting, restraining, slowing, stopping, or reversing the progression or severity of a pathological condition, or sequela thereof, described herein. The term "preventing" means reducing the likelihood that the recipient of a compound of formula I will incur, further incur or develop any of the pathological conditions, or sequela thereof, described herein.

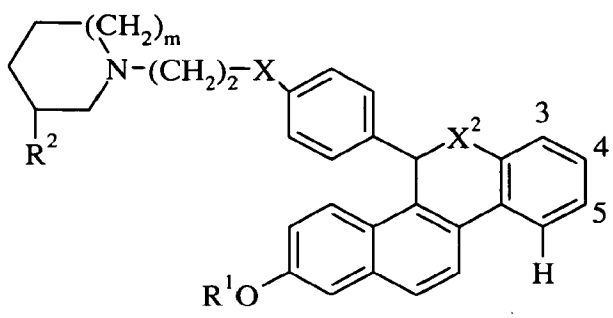
The term "a patient in need thereof" is a patient either suffering from the caimed pathological condition or sequela thereof or is a patient at a recognized risk thereof as determined by medical diagnosis, *i.e.*, as determined by the attending physician.

As used herein, the term "effective amount" means an amount of a compound of formula I that is capable of treating the conditions described herein.

Preferred Compounds and Embodiments of the Invention

Certain compounds of the invention are particularly interesting and are preferred. The following listing sets out several groups of preferred compounds. It will be understood that each of the listings may be combined with other listings to create additional groups of preferred compounds. The following numbering systems will be used to describe the preferred positions of the $COHR^3R^{3a}$ moiety:





a) m is 1 or 2;

b) m is 1;

c) R^1 is H;

d) R^1 is H or COR^4 and R^4 is C_1-C_6 alkyl, $NHCH_3$ or phenyl;

e) R^1 is H or COR^4 and R^4 is C_1-C_4 alkyl, $NHCH_3$ or phenyl;

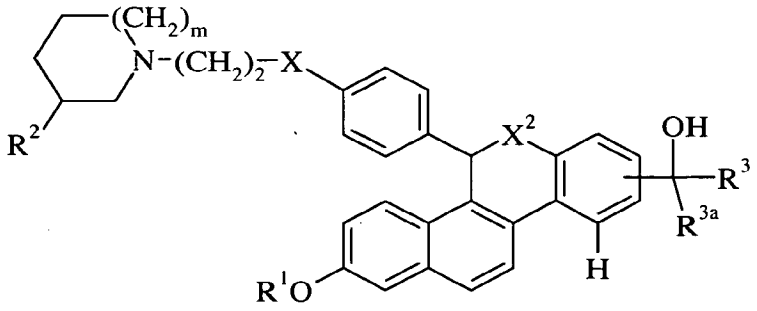
f) R^3 and R^{3a} are independently H or C_1-C_4 alkyl;

g) R^3 and R^{3a} are independently H or methyl;

h) the $COHR^3R^{3a}$ moiety is at position 4;

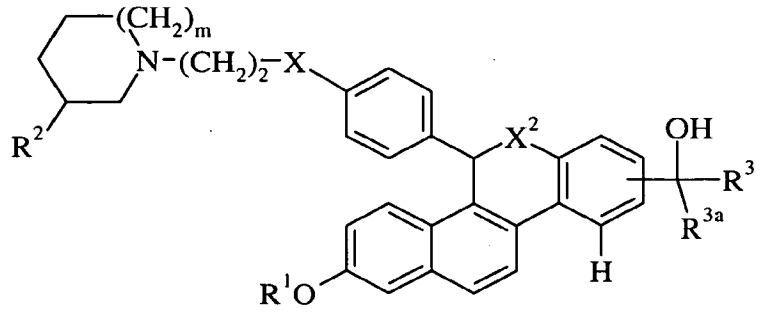
i) R is H;

j) R combines with X^1 to form a moiety of the formula:



;

k) R combines with X^1 to form a moiety of the formula:



and X^2 is O;

l) X is O;

m) X is NR^5 and R^5 is H or methyl;

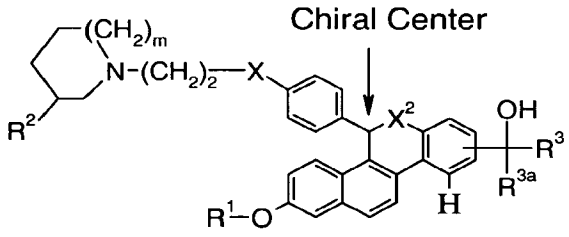
n) X^1 is O or CH_2;

o) X^1 is O;

p) Y is CH=CH;

q) the hydrochloride salt form.

With respect to the chiral center designated below:



an enantiomeric excess (ee) of greater than 90% is preferred, an ee of greater than 95% is most preferred and an ee of greater than 99% is most especially preferred. Enantiomeric enrichment is readily determined by one of ordinary skill in the art using standard techniques and procedures, such as gas or high performance liquid chromatography with a chiral column (see, e.g., J. Jacques, et al., "Enantiomers, Racemates, and Resolutions", John Wiley and Sons, Inc., 1981; E.L. Eliel and S.H. Wilen," Stereochemistry of Organic Compounds", (Wiley-Interscience 1994), and European Patent Application No. EP-A-838448, published April 29, 1998). Of course, the preferred enantiomer is that which possesses favorable activity in the biological assays disclosed herein. In order to verify

the identify of the preferred enantiomer in any given racemic mixture, the activity of the individual isomers should be verified in the biological assays described herein.

The preferred patient of treatment is a female human.

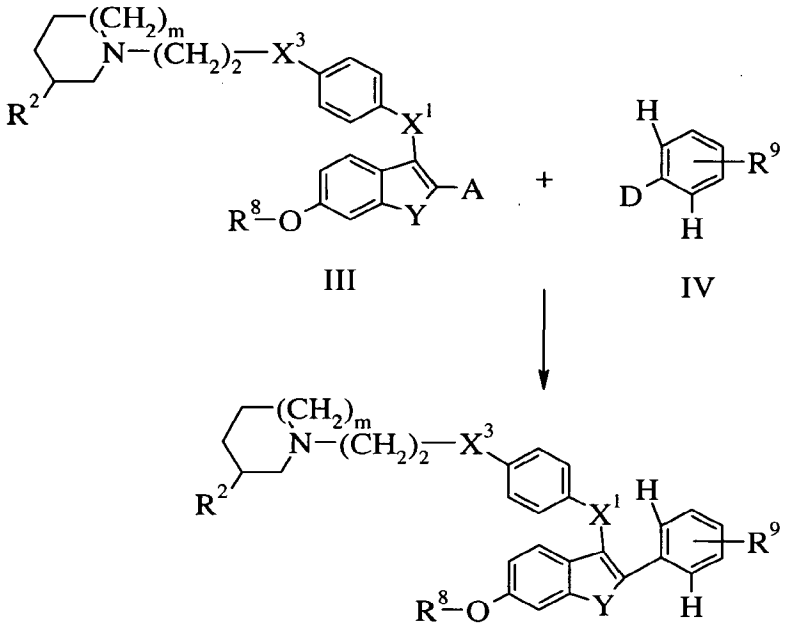
The compound of formula I is preferably formulated in a dosage unit form, *i.e.*, in an individual delivery vehicle, for example, a tablet or capsule, prior to administration to the recipient woman.

The compound of formula I is preferably administered orally.

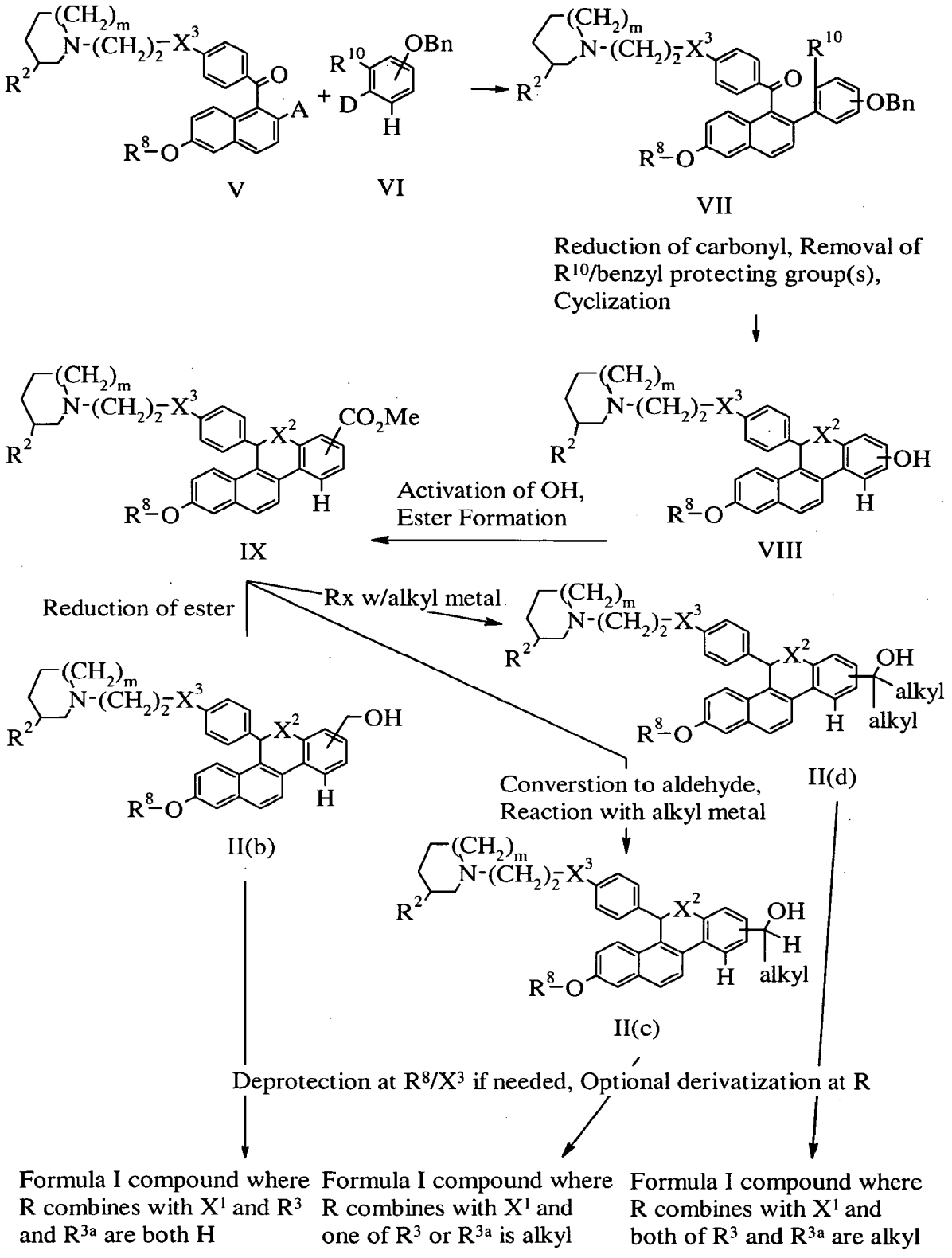
Synthesis

The compound of formula I may be prepared as described in the following Schemes and Examples.

Scheme 1



Scheme 2


V
VI
VII
Reduction of carbonyl, Removal of R10/benzyl protecting group(s), Cyclization
VIII
Activation of OH, Ester Formation
IX
Reduction of ester
Rx w/alkyl metal
II(b)
II(d)
Converstion to aldehyde, Reaction with alkyl metal
II(c)
Deprotection at R8/X3 if needed, Optional derivatization at R
Formula I compound where R combines with X1 and R3 and R3a are both H
Formula I compound where R combines with X1 and one of R3 or R3a is alkyl
Formula I compound where R combines with X1 and both of R3 and R3a are alkyl

In Scheme 1, where R^9 is CH_2OH or $C(O)C_1$-C_6 alkyl, the synthesis of a compound of formula I where R is H is illustrated. A compound of formula IV is reacted with a compound of formula III under usual "Suzuki" or "Stille" reaction conditions, *i.e.*, wherein one of substituent "A" or "D" is a boronic acid/ester or alkyl stannane moiety and the other is a leaving group, *e.g.*, chloro, bromo or iodo or a sulfonate group such as trifluoromethyl sulfonate to form a compound of formula II(a). A compound of formula I where R^3 and R^{3a} are both hydrogen, where one of R^3 and R^{3a} is alkyl and where both of R^3 and R^{3a} are alkyl may be accessed as illustrated in the Scheme and as taught below in the working examples.

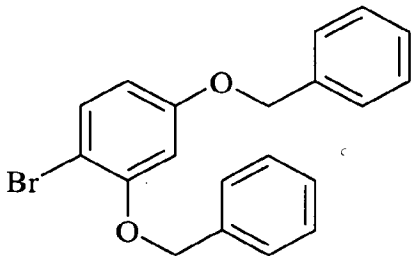
In Scheme 2, where R^{10} is alkyl or benzyl protected thio or hydroxy and "Bn" denotes benzyl, a compound of formula V is reacted with a compound of formula VI under usual "Suzuki" or "Stille" reaction conditions as described above to form a compound of formula VII. The ketone in the formula VII compound may then be reduced to the corresponding alcohol employing typical procedures for such a transformation (see working examples below). The benzyl protecting group along with the hydroxy or thio protecting group at R^{10} may then be removed under conditions that also promote cyclization (see working examples below) to provide the compound of formula VIII. The free hydroxy group found in the compound of formula VIII may then be activated towards nucleohilic displacement, e.g., by formation of the triflate. Said activated hydroxy compound may then be reacted with carbon monoxide under transition metal catalysis (*e.g.*, $Pd(Oac)_2$) in the presence of methanol to afford the corresponding methyl ester. Said ester may then be reduced under standard conditions (*e.g.*, with $LiAlH_4$) to form the compound of formula II(b). A compound of formula I or II where only one of R^3 or R^{3a} is alkyl may be prepared by reacting the aforementioned ester with DIBAL to yield the corresponding aldehyde, followed by reaction with at least one equivalent of an alkyl metal (*e.g.*, alkyl lithium). A compound of formula I or II where R^3 and R^{3a} are alkyl may be prepared by reacting the aforementioned ester with at least two equivalents of an alkyl metal (*e.g.*, alkyl lithium).

When R^8 in the formula II(a) and II(b) compounds is SO_2CH_3, C_1-C_6 alkyl or benzyl (preferably methyl, benzyl or SO_2CH_3) said hydroxy protecting groups may be

removed under standard conditions (see, *e.g.*, the procedures that follow or the latest edition of Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, N.Y.) to provide the compound of formula I where R^1 is H. Similarly, when X^3 is NR^9 and R^9 is $CO_2(C_1\text{-}C_6$ alkyl), said amino protecting group may also be removed as taught in Greene. A formula I compound where R^1 is H may be further derivatized employing standard acylation or sulfonylation methodology to prepare a compound of formula I where R^1 is COR^4 or or $SO_2(n\text{-}C_4\text{-}C_6$ alkyl).

Preparation 1

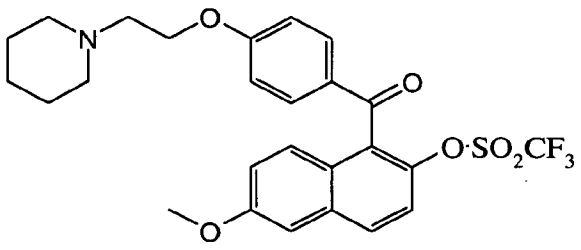
2,4-Bisbenzyloxy bromobenzene



Charge a 1 liter flask with 500 ml dry dimethylformamide (DMF) and add 4-bromoresorcinol (9.5 grams, 0.05 moles) and start stirring. To this mixture add sodium hydride (60 % in oil, 6 grams, 0.15moles) in portions over ½ hour. To this mixture add benzyl bromide (29 grams, 0.168 moles) in portions over ½ hour. After two hours the reaction is complete as determined by TLC (silica gel, methylene chloride/hexane 1:1). Quench the reaction with ammonium chloride solution and remove the solvent on a rotavap at 80 degrees at which point the reaction mixture turns deep purple. Dissolve this in methylene chloride and wash three times with water, then 0.1 N sodium hydroxide then brine. Dry the solvent over 3A molecular sieves. Run the dark purple solution through a short plug of silica gel, which removes the purple color. Evaporate the filtrate to an oil and purify on a Biotage silica gel flash column, eluting excess benzyl bromide with 10 % methylene chloride/hexane then elute the product with 20 % methylene chloride/hexane. Evaporate the solvent to an oil, add methanol and chill overnight. In the morning filter the white crystals and air dry to give 11.2 g of 2,4-bisbenzyloxy bromobenzene (66%).

Preparation 2

Trifluoromethanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester



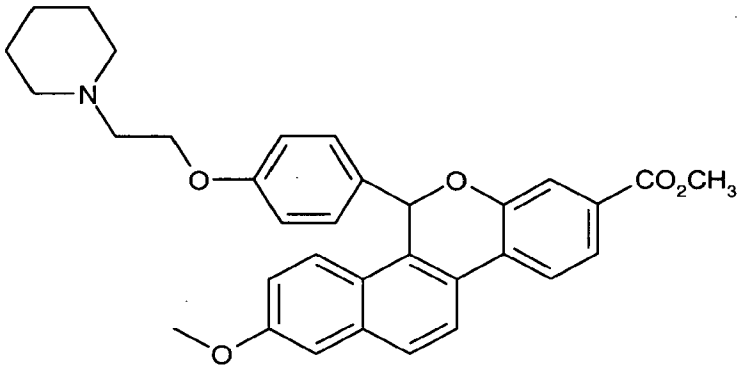
Dissolve 2,6-dimethoxynaphthalene (1.0 eq) in CH_2Cl_2 (5 volume equivalents) at ambient temperature in a dry round bottom flask equipped with stir bar, temperature probe and N_2 line. Cool the solution to 0 °C with an ice bath, and add 4-(2-piperidin-1-yl-ethoxy)-benzoyl chloride (1.1 eq). Add aluminum chloride (2.0 eq). Once the reaction is determined to be complete, quench the reaction slowly with 1 N NaOH and dilute with additional water and CH_2Cl_2. Wash the aqueous layer with CH_2Cl_2 (20 mL). Combine the organic extracts and wash with brine and dry (Na_2SO_4). Recrystallize the crude product from methanol to give (2,6-dimethoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone.

Dissolve (2,6-dimethoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone in CH_2Cl_2 (10 volume equivalents) in a 3-neck round bottom flask equipped with a pressure equalizing addition funnel, stirbar, and N_2 source. Cool the flask in an ice/brine bath and add 1.0 M BCl_3 solution in CH_2Cl_2 (1.2 equivalents) dropwise. The reaction solution turns dark red and the temperature initially increases to 5 °C. After about 1 hour, quench the reaction with methanol (5 equivalents) and allow to warm to room temperature. Dilute the organic solution with CH_2Cl_2 (one volume equivalent) and add a 1.0 M $NaHCO_3$ solution (5 volume equivalents) and stir for one hour. Separate the aqueous and organic layers. Wash the aqueous layer with CH_2Cl_2 (one volume) and combine the organic layers. Wash with saturated NH_4Cl and dry over Na_2SO_4. Purify the product via column chromatography (50/1 silica gel) eluting with CH_2Cl_2/hexanes (3/1) to yield (2-hydroxy-6-methoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone.

Dissolve (2-hydroxy-6-methoxy-naphthalen-1-yl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone in CH_2Cl_2 (10 volumes) in a three neck round bottom flask equipped with a stir bar and N_2 source and chill to 0°C in an ice/brine bath. Add pyridine (1.3 equivalents). Add trifluoromethanesulfonyl chloride (1.2 equivalents) via syringe over 15 minutes. After about 15 minutes, quench the reaction with H_2O (10 volumes), wash with 1 N aqueous HCl (5 volumes) and 1.0 N aqueous $NaHCO_3$, and dry over Na_2SO_4. Obtain the title compound in quantitative yield after concentration.

Preparation 3

2-Methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysene-8-carboxylic acid methyl ester



Dissolve trifluoromethanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-benzoyl]-naphthalen-2-yl ester, (1.89 grams, 3.52 mmoles) in 100 ml acetonitrile and add to a flask containing bis(pinacolato)diboron (1.07 grams, 4.23 mmoles), palladium acetate (79 mg. 0.35 mmoles), triphenylphosphine (185 mg. 0.70 mmoles) and cesium fluoride (1.6 grams, 10.56 mmoles). Heat and stir the mixture under nitrogen for two hours at reflux. Cool the reaction slightly and add 2,4 bis(benzyloxy)bromobenzene (2.6 grams, 7.0 mmoles) along with another portion of the diboron, palladium acetate, and triphenylphosphine. Continue refluxing for 24 hours. Cool the mixture, filter off the solids and run the filtrate through an SCX column. Wash the columns with methanol and elute with 2N ammonia in methanol. Evaporate the filtrate to give 1.8 grams of a dark brown gum. Purify on a flash column using silica gel eluting with a gradient of 0 to 5% methanol in methylene chloride. Evaporate the solvent to yield 1.1 gram of [2-(2,4-bis-

benzyloxyphenyl)-6-methoxynaphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone (46%).

Dissolve [2-(2,4-Bis-benzyloxyphenyl)-6-methoxynaphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]methanone (1.1 grams, 1.6 mmoles) in 10 ml of tetrahydrofuran (THF) and add a 1.0 molar solution of lithium aluminum hydride (5 ml. 5.0 mmoles). Stir for 30 minutes at which time the reaction is complete as determined by LC/MS. Quench the reaction with sodium bicarbonate solution and extract with a 3/1 mixture of chloroform and isopropanol. Acidify the water layer to pH=7.0 and extract again. Combine the organic layers and dry over 3A molecular sieves. Evaporate the solvent to give 1.0 g of [2-(2,4-bis-benzyloxy-phenyl)-6-methoxynaphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanol.

Dissolve [2-(2,4-bis-benzyloxy-phenyl)-6-methoxynaphthalen-1-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanol (900 mg., 1.32 mmoles) in 250 ml of THF and add 20 ml of 5N HCl and 700 mg 10% Pd/C (slurried in THF). Place the reaction mixture under a balloon of nitrogen and stir for 24 hours. Filter the reaction mixture and add saturated sodium bicarbonate. Extract the aqueous phase 2 times with a 3/1 mixture of chloroform and isopropanol. Dry the organic layer over 3A molecular sieves, evaporate and triturate the resulting gum with ether to give 521 mg of 2-methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-8-ol (82%).

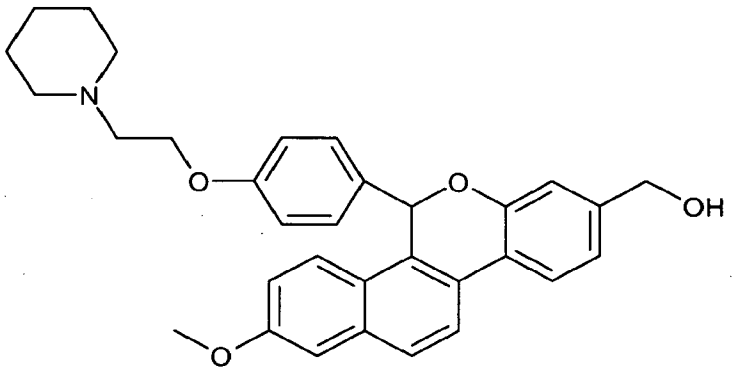
Dissolve 2-methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-8-ol (96 mg. 0.2 mmoles) in 10 ml. of methylene chloride and add N,N-bis(trifluoromethylsulfonyl)aniline (92 mg. 0.25 mmoles) followed by diisopropylethyl amine (32 mg., 0.25 mmoles). Stir for 2 hours and check by LC/MS. Still much starting material left so another portion of the aniline and amine were added. After 2 hours still considerable starting material left, so add 500 microliters of the amine and leave stand overnight. In the morning the reaction was complete. Rotavap the solvent, dissolve the residue in methanol and run through and SCX column, eluting with 2N ammonia in methanol. Evaporate the solvent to give 74 mg of trifluoromethanesulfonic acid 2-methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-8-yl ester (61%).

Dissolve trifluoromethanesulfonic acid 2-methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-8-yl ester (110 mg 0.179 mmoles) in 25 ml. of methanol and add 0.1 equivalent of palladium acetate, 0.1 equivalent of diphenylphosphinobutane and

2.2 equivalents of triethyl amine. React in a high pressure vessel with carbon monoxide at 1000 psi and 110 degrees. Purify product by running it through an SCX column and eluting with 2 N ammonia in methanol to give 46 mg of the title compound (43%).

Example 1

{2-Methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-8-yl}-methanol



Dissolve 2-methoxy-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysene-8-carboxylic acid methyl ester (46 mg. 0.09 mmoles) in 50 ml. THF and add 5 ml of 1.0 molar lithium aluminum hydride solution in THF. Stir for 30 minutes and check for completeness. Quench with sodium bicarbonate and extract the water layer two times with a 3/1 mixture of chloroform and isopropanol. Dry the solvent and evaporate to a glass. This material was used in the next step without purification.

Example 2

8-Hydroxymethyl-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-2-ol

Dissolve the compound of Example 1 in 25 ml. DMF and add excess sodium t-butyllthiolate. Heat the mixture under nitrogen at 110°C for 18 hours. Neutralize the mixture with acetic acid and evaporate to a paste. Dissolve the material in methanol and purify on an SCX column, eluting with 2 N ammonia in methanol to give 26 mg of the title compound (62%). 1H-NMR (CD3OD, 400.00 MHz): 7.93 (d, J = 8.8 Hz, 1H); 7.77 (dd, J = 8.4, 3.2 Hz, 2H); 7.63 (d, J = 9.2 Hz, 1H); 7.17 (d, J = 2.8 Hz, 1H); 7.08 (s, 1H); 7.05 (d, J = 3.6 Hz, 2H); 7.03 (d, J = 2.8 Hz, 1H); 6.98 (s, 1H); 6.97-6.96 (m, 1H); 6.84

(d, J = 1.2 Hz, 1H); 6.72 (s, 1H); 6.71 (d, J = 12.0 Hz, 1H); 4.50 (s, 2H); 4.02-3.99 (t, 2H);
2.74-2.71 (t, 2H); 2.52 (m, 4H); 1.63-1.57 (m, 5H); 1.48-1.44 (m, 2H)

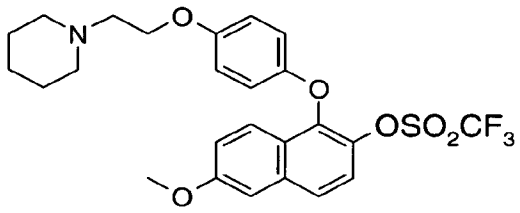
Examples 3 and 4

8-Hydroxymethyl-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysen-2-ol

The racemic mixture from Example 2 was separated on a Chiralpak AD column using 40% isopropanol/heptane mixture on a 0.46 x 25 cm column eluting at 1.0 ml/min. and monitoring at 225 nm. The compound that elutes first is Example 3 and the second eluting compound is Example 4.

Preparation 4

Trifluoromethanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl ester



Add 6-methoxynaphthalene-2-ol (20 g, 114.8 mmol) to dimethylformamide (DMF, 250 mL) at ambient temperature followed by *N*-bromosuccinimide (NBS, 21.5 g, 120 mmol) over a 30 minute period. After 45 minutes, dilute with water (800 mL), collect and dry the precipitate to provide 25.5 g (87%) of 1-bromo-6-methoxy-naphthalen-2-ol.

Add 1-bromo-6-methoxy-naphthalen-2-ol (66.7 g, 264 mmol), potassium carbonate (K_2CO_3, 40.0 g, 290 mmol) and benzyl bromide (49.6 g, 290 mmol) to DMF (800 mL). Stir the mixture at ambient temperature for 1 hour. Add water (400 mL) to precipitate the product. Collect the precipitate and wash the filter cake with heptane (3 X 125 mL) then dry to provide 83.7 g of 2-benzyloxy-1-bromo-6-methoxy-naphthalene (86.2%).

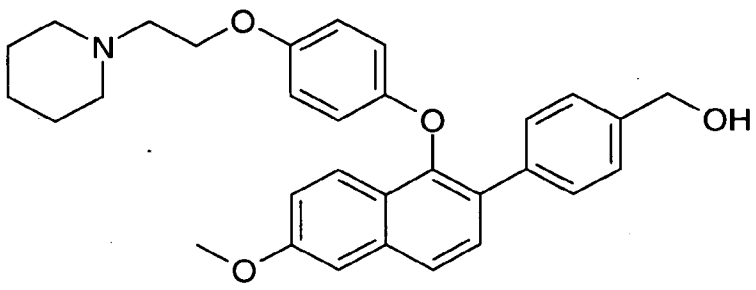
Combine toluene (200 mL), 2-benzyloxy-1-bromo-6-methoxy-naphthalene (30 g, 87.4 mmol), 4-(2-piperidin-1-yl-ethoxy)phenol (23.2 g, 105 mmol) and cesium carbonate (34.4 g, 105 mmol), and heat the mixture to reflux. Remove a portion of the toluene (100 mL). Add ethyl acetate (390 mg, 4.37 mmol) and copper triflate benzene complex (2.20 g, 4.37 mmol) to the reaction mixture and stir for 5 minutes. Remove the solvent by distillation and heat the resulting residue to 174°C for 1.5 hours. Dissolve the residue in a mixture of ethyl acetate (200 mL) and aqueous HCl (1 N, 90 mL). Separate and concentrate the organics to a residue. Column chromatograph the residue to give 12.4 g of 1-{2-[4-(2-benzyloxy-6-methoxy-naphthalen-1-yloxy)-phenoxy]-ethyl}-piperidine (30%).

Add 1-{2-[4-(2-benzyloxy-6-methoxy-naphthalen-1-yloxy)-phenoxy]-ethyl}-piperidine (12.4 g, 25.5 mmol) to a methanol/ethyl acetate mixture (1:1, 490 mL) and heat to form a solution. Remove the heat and add ammonium formate (4.83 g, 76.6 mmol) and $Pd(OH)_2$ on carbon (20 % ww, 1.58 g, 1.12 mmol). Reflux for 50 minutes then filter the mixture. Concentrate the filtrate to provide 9.9 g of 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalene-2-ol (98.5%).

Cool dichloromethane (290 mL), triethylamine (3.08 g, 30.4 mmol) and 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalene-2-ol (9.2 g, 23.4 g) to -50°C and add trifluoromethane sulfonic acid anhydride (7.26 g, 25.7 mmol). Stir the resulting mixture at -50°C for 2 hours then allow the mixture to warm to ambient temperature before stirring for an additional hour. Add brine (150 mL) and separate the organics. Wash the organics with $NaHCO_3$ then dry before concentrating to a residue. Crystallize the residue with ethyl ether – hexanes to provide 11.2 g of the title compound (90.9%).

Example 5

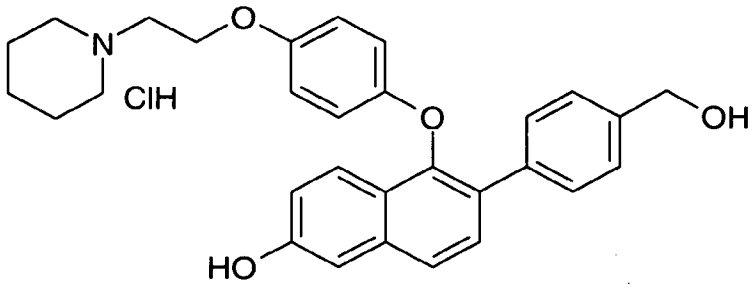
(4-{6-Methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-methanol



Combine trifluoromethanesulfonic acid 6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl ester (500 mg, 0.95 mmol), 4-hydroxymethylphenylboronic acid (435 mg, 2.85 mmol), K_2CO_3 (530 mg, 3.8 mmol), LiCl (160 mg, 3.8 mmol), toluene (10 ml), and water (1 ml), stir and bubble nitrogen into the slurry for 3 minutes. Add the catalyst, [1,1'-bis(diphenylphosphino)ferrocene] dichloropalladium(II), complex with dichloromethane (1:1) (390 mg, 0.48 mmol, 0.5 eq.), to the reaction mixture and heat to 90°C. After 18 hours, cool the reaction mixture to ambient temperature and dilute with diethyl ether (50 ml) and water (10 ml). Filter through a pad of celite and separate the layers. Wash the organic layer with brine (10 ml), dry with Na_2SO_4, filter, and concentrate in vacuo. Chromatograph the residue on a SiO_2 column eluting the material with methanol (0 to 7.5%) in dichloromethane to 10% methanol in dichloromethane (containing 0.5% NH_4OH) to give 321 mg (70%) of the title compound: Mass spectrum (ion spray): m/z = 484.5 (M+H).

Example 6

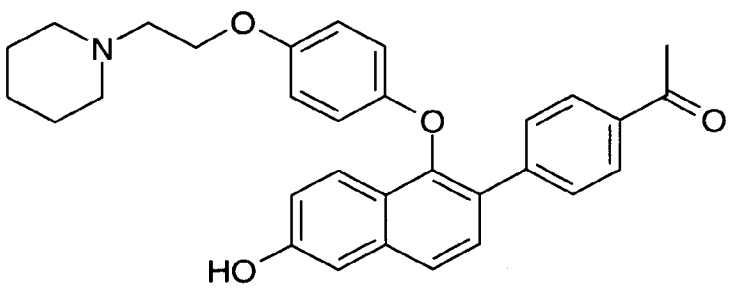
6-(4-Hydroxymethyl-phenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-ol, hydrochloride



Combine (4-{6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-methanol (234 mg, 0.48 mmol), sodium ethanethiol (135 mg, 1.6 mmol), and N,N-dimethylformamide (10 mL). Heat solution to 160□C for 8 hours. Cool the reaction mixture to ambient temperature and dilute with water (70 mL) and ethyl acetate (50 mL). Separate the layers and extract the aqueous layer with ethyl acetate (50 mL). Combine the organic layers, dry with Na_2SO_4, filter, and concentrate in vacuo. Chromatograph the residue on a SiO_2 column eluting the material with methanol in dichloromethane (0 to 14%) to give 135 mg of the free base of the title compound. Dissolve the free base in ethyl acetate (2 mL) and methanol (0.2 mL) and dilute with diethyl ether (5 mL). Cool in an ice bath and treat with 2M HCl in diethyl ether (0.22 mL, 0.44 mmol). Dilute the reaction mixture with diethyl ether (25 mL) and collect the solid on filter paper. Rinse with diethyl ether and dry at 65°C for 48 hours in vacuo (<2mm of Hg) to give 124 mg (85%) of the title compound: Mass spectrum (ion spray): m/z = 470.5 (M+H-HCl).

Example 7

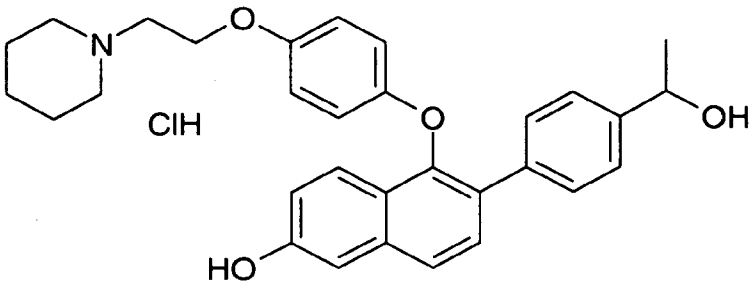
1-(4-{6-Hydroxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-ethanone



Combine trifluoro-methanesulfonic acid 6-methoxyoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl ester (1 g, 1.9 mmol), 4-acetylphenylboronic acid (0.94 mg, 5.7 mmol), CsF (2.6 g, 17.1 mmol), and acetonitrile (20 ml). Add [1,1'-bis(diphenylphosphino)ferrocene] dichloropalladium(II), complex with dichloromethane (1:1) (775 mg, 0.95 mmol, 1 eq.) to the reaction mixture and heat to 90°C. After 22 hours, cool the reaction mixture to ambient temperature and evaporate the solvent. Dilute with diethyl ether (100 ml) and sonicate the mixture for 10 minutes. Filter through a pad of celite and concentrate the filtrate in vacuo. Chromatograph the residue on a SiO_2 column eluting the material with methanol in dichloromethane (0 to 6%) to give 844 mg (89%) of 1-(4-{6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-ethanone: Mass spectrum (ion spray): m/z = 496.6 (M+H).

Combine 1-(4-{6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-ethanone (576 mg, 1.16 mmol) and pyridinium hydrochloride (7.5 g, 65 mmol) and heat to 200°C. Every 15 minutes add additional pyridinium hydrochloride (1 g) and monitor the reaction by mass spectroscopy. After 1.25 hours, cool the reaction mixture to ambient temperature and dissolve the residue in saturated aqueous $NaHCO_3$ (100 mL), ethyl acetate (250 mL) and methanol (10 mL). Separate the layers and extract the aqueous layer with a mixture of methanol (5 mL) and ethyl acetate (100 mL). Combine the organic layers, wash with water (50 mL), dry with Na_2SO_4, filter and concentrate. Triturate the crude reaction material with ethyl acetate (60 mL), filter away the solids and concentrate the filtrate. Chromatograph the residue on a SiO_2 column eluting with methanol in dichloromethane (0 to 10%) to give 317 mg (57%) of the title compound: Mass spectrum (ion spray): m/z = 482.5 (M+H).

Example 8

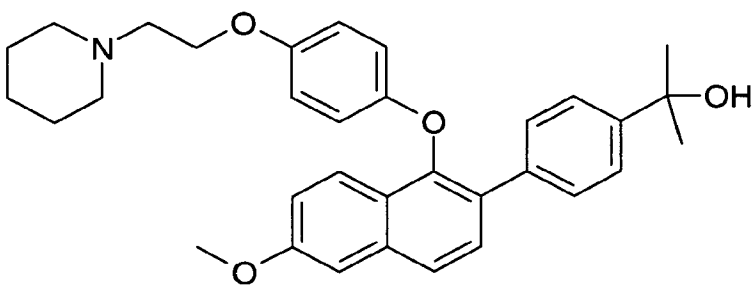


6-[4-(1-Hydroxy-ethyl)-phenyl]-5-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-ol hydrochloride

Dissolve 1-(4-{6-hydroxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-ethanone (162 mg, 0.34 mmol) in THF (20 mL) and cool in an ice bath. Add 1M LAH in THF (0.9 mL, 0.9 mmoL) and stir for 1 hour. Sequentially add water (150 mL), 15% aqueous NaOH (35 mL), and ethyl acetate (40 mL) to the reaction mixture. Filter the slurry through packed celite and separate the biphasic filtrate. Wash the organic layer with water (2 X 5 mL) and brine (5 mL), dry with Na_2SO_4, filter, and concentrate in vacuo to obtain 146 mg of the free base of the title compound. Dissolve in ethyl acetate (2 mL) and dilute with diethyl ether (20 mL). Cool in an ice bath and treat with 2M HCl in diethyl ether (0.17 mL, 0.34 mmol). Dilute the reaction mixture with diethyl ether (25 mL) and collect the solid on filter paper. Rinse with diethyl ether and dry at 65°C for 48 hours in vacuo (<2mm of Hg) to give 94 mg (54%) of the title compound: Mass spectrum (ion spray): m/z = 466.5 (M+H-HCl-H_2O).

Example 9

2-(4-{6-Methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-propan-2-ol



Dissolve 1-(4-{6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-ethanone (300 mg, 0.6 mmol) in diethyl ether (25 mL) and treat dropwise with a 1.4M solution of methyl magnesium bromide (1.9 mL, 4 mmol). Stir for 24 hours and then slowly quench with saturated aqueous ammonium chloride (25 mL). Dry the organic layer with Na_2SO_4, filter, and concentrate in vacuo. Chromatograph the residue on a SiO_2 column eluting the material with methanol in dichloromethane (0 to 6%) to give 306 mg (75%) of the title compound: ^{1}H NMR ($CDCl_3$): 7.86 (d, 1H), 7.68 (d, 1H), 7.55 (d, 1H), 7.51-7.54 (m, 2H), 7.42-7.45 (m, 2H), 7.19 (d, 1H), 7.08 (dd, 1H), 6.60-6.68 (m, 4H), 3.98-4.04 (m, 2H), 3.94 (s, 3H), 2.72-2.78 (m, 2H), 2.48-2.58 (m, 4H), 1.57-1.67 (m, 4H), 1.57 (s, 6H), 1.41-1.48 (m, 2H).

Example 10

6-[4-(1-Hydroxy-1-methyl-ethyl)-phenyl]-5-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-ol hydrochloride

Combine 2-(4-{6-methoxy-1-[4-(2-piperidin-1-yl-ethoxy)-phenoxy]-naphthalen-2-yl}-phenyl)-propan-2-ol (230 mg, 0.45 mmol), sodium ethanethiol (190 mg, 2.2 mmol), and N,N-dimethylformamide (10 mL). Heat solution to 160°C for 1 hour. Cool the reaction mixture to ambient temperature and dilute with water (70 mL) and ethyl acetate (50 mL). Separate the layers and extract the aqueous layer with ethyl acetate (50 mL). Combine the organic layers, wash with brine (50 mL), dry with Na_2SO_4, filter, and concentrate in vacuo. Chromatograph the residue on a SiO_2 column eluting the material with methanol in dichloromethane (0 to 15%) to give 198 mg of the free base of the title compound. Dissolve the free base in ethyl acetate (2 mL) and dilute with diethyl ether (10 mL). Cool in an ice bath and treat with 2M HCl in diethyl ether (0.3 mL, 0.6 mmol) to obtain an off-white solid. Dilute the reaction mixture with diethyl ether (25 mL) and collect the solid on filter paper. Rinse with diethyl ether and dry at 65°C for 48 hours in vacuo (<2mm of Hg) to give 90 mg (37%) of the title compound: Mass spectrum (ion spray): m/z = 480.3 (M+H-H_2O-HCl).

Formulation

Because the free base form of a compound of formula I contains a basic moiety (*i.e.*, amino), said compound may be formulated as a pharmaceutical acid addition salt, *e.g.*, as the hydrochloride salt or as a salt described in "Handbook of Pharmaceutical Salts: Properties, Selection and Use", Weinheim, New York: VHCA; Wiley-VCH, 2002.

The present pharmaceutical compositions are prepared by known procedures using well-known and readily available ingredients. In making the formulations of the present invention, the active ingredient (formula I compound) will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents.

Biological Assays

Estrogen Receptor Binding Assay: Representative compounds of the present invention are screened for binding affinity to both estrogen receptor types (ERα and ERβ). This competition binding assay measures the compound's ability to displace ^{3}H-estradiol and generates IC_{50} and K_i values for both receptor types.

This competition binding assay is run in a buffer containing 50mM Hepes, pH 7.5, 1.5mM EDTA, 150mM NaCl, 10% glycerol, 1mg/mL ovalbumin and 5mM DTT, using 0.025 μCi per well ^{3}H-Estradiol(NEN #NET517 at 118 Ci/mmol, 1 mCi/mL), 10 ng/well ERAlpha or ERbeta receptor (PanVera). A compound of the present invention is added at 10 different concentrations. Non-specific binding is determined in the presence of 1μM of 17-B Estradiol. The binding reaction (140 μl) is incubated for 4 hours at room temperature, then 70 μl of cold DCC buffer is added to each reaction (DCC buffer

contains per 50 mL of assay buffer, 750 mg of charcoal (Sigma) and 250 mg of dextran (Pharmacia)). Plates are mixed 8 minutes on an orbital shaker at 4°C. Plates are then centrifuged at 3,000 rpm at 4°C for 10 minutes. An aliquot of 120 µl of the mix is transferred to another 96-well, white flat bottom plate (Costar) and 175 µl of Wallac Optiphase "Hisafe 3" scintillation fluid is added to each well. Plates are sealed and shaken vigorously on an orbital shaker. After an incubation of 2.5 hours, the plates are read in a Wallac Microbeta counter. The data is used to calculate an IC_{50} and % Inhibition at 10µM. The K_d for ^{3}H-Estradiol is determined by saturation binding to ER alpha and ER beta receptors. The IC_{50} values for test compounds are converted to K_i using Cheng-Prusoff equation and the K_d determined by saturation binding assay.

Ishikawa Cell Proliferation Assay: This assay measures cell proliferation (using an alkaline phosphatase readout) in both an agonist mode in the presence of a compound of the present invention alone, and in an antagonist mode in which the ability of a compound of the present invention to block estradiol stimulation of growth is measured.

Ishikawa human endometrial tumor cells are maintained in MEM (minimum essential medium, with Earle's salts and L-Glutamine, Gibco BRL, Gaithersburg, MD), supplemented with 10% fetal bovine serum (FBS) (V/V), (Gibco BRL). One day prior to assay, growth media is changed to assay medium, DMEM/F-12 (3:1) (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, 3:1 Mixture, phenol red-free, Gibco BRL) supplemented with 5% dextran coated charcoal stripped fetal bovine serum (DCC-FBS) (Hyclone, Logen, UT), L-Glutamine (2mM), MEM sodium pyruvate (1 mM), HEPES (N-[2-hydroxyethyl]piperazine-N' – [2-ethanesulfonic acid] 2 mM) all from Gibco BRL). After an overnight incubation, Ishikawa cells are rinsed with Dulbecco's Phosphate Buffered Saline (1X) (D-PBS) without Ca^{+2} and Mg^{+2} (Gibco BRL), and trypsinized by a 3 minute incubation with 0.25% Trypsin/EDTA, phenol red-free (Gibco BRL). Cells are resuspended in assay medium and adjusted to 250,000 cells/mL. Approximately 25,000 cells in a 100ul media are added to flat-bottom 96 wells microculture plates (Costar 3596) and incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours. The next day, serial dilutions of compounds are prepared in assay medium (at 6 times the final concentration in the assay). The assay is run in dual mode, agonist and antagonist modes.

For the agonist mode, plates receive 25 µl/well of assay medium followed by 25 µl/well of a diluted compound of the present invention (at 6x the final concentrations). For the antagonist mode, plates receive 25 µl/well of 6 nM E_2 (β-Estradiol, Sigma, St. Louis, MO) followed by 25 µl/well of a diluted compound of the present invention (at 6x the final concentrations). After an additional 48-hour incubation at 37°C in a 5% CO_2 humidified incubator, media is aspirated from wells and 100 µl fresh assay medium is added to each microculture. Serial dilutions of compounds are prepared and added to the cells as described above. After an additional 72 hour incubation at 37°C in a 5% CO_2 humidified incubator, the assay is quenched by removing media and rinsing plates twice in Dulbecco's Phosphate Buffered Saline (1X) (D-PBS) (Gibco BRL). The plates are dried for 5 minutes and frozen at -70°C for at least 1 hour. The plates are then removed from the freezer and allowed to thaw at room temperature. To each well, 100 µl of 1-Step™ PNPP (Pierce Chemical Company, Rockford, IL) is added. After a 20-minute incubation, plates are read on a spectophotometer at 405nm.

The data is fitted to a linear interpolation to derive EC_{50} (for agonist mode) or IC_{50} (for antagonist mode) values. For the antagonist mode, a % efficacy for each compound is calculated versus E2 (1nM) alone. For the agonist mode, a % efficacy for each compound is calculated versus the response to tamoxifen.

In the agonist mode, the compounds of Examples 2, 3, 4, 6, 8 and 10 were tested and were found to be less stimulatory than tamoxifen. For example, the compound of Example 2 had a relative % efficacy of 25%. In the antagonist mode, these same compounds inhibited greater than at least 80% of the 1nM estradiol response. For example, the compound of Example 2 had an IC_{50} of 36 nM and a % efficacy of 92%.

MCF-7 Proliferation Assay: The MCF-7 cell line was derived from a human breast adenocarcinoma and is used as an indicator of potential antiproliferative activity in breast epithelium.

MCF-7 breast adenocarcinoma cells (ATCC HTB 22) are maintained in MEM (minimal essential medium, phenol red-free, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (V/V), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES ((N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]10 mM}, non-essential amino acids(0.1mM)and Penicillin Streptomycin(1X). Seven days prior to assay, MCF-7 cells

are switched to assay media which is the same as maintenance medium except supplemented with 10% dextran-coated charcoal-stripped fetal bovine serum (DCC-FBS) assay medium in place of 10% FBS. MCF-7 cells are removed from flasks using 10X Trypsin EDTA (phenol red free, Gibco BRL) and diluted to 1X in (Ca++/Mg++ free HBSS (phenol red-free). Cells are adjusted to 80,000 cells/mL in assay medium. Approximately 8,000 cells (100 µl) are added to each well in 96 well Cytostar T scintillation plates (Amersham) and incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours to allow cell adherence and equilibration after transfer.

Serial dilutions of a compound of the present invention are prepared in assay medium at 4x the final desired concentration). A 50 µl aliquot of test compound dilutions (at 4x the final assay concentration) is transferred to duplicate wells followed by 50 µl assay medium for the agonist mode or 50 µl of 40pM of E2 for the antagonist mode to a final volume of 200 µl. For each of the agonist plates, a basal level (media) and a maximum stimulated level (with 1µM E2) is determined. For each of the antagonist plates, a basal level (media) and an E2 (10pM) alone control is determined. After an additional 48 hours at 37°C in a 5% CO_2 humidified incubator, 20µl of assay medium containing 0.01 µCi of ^{14}C-thymidine (52 mCi/mmol, 50 µCi/µl, Amersham) is added to each well. The plates are incubated overnight in the same incubator and then counted on the Wallac Microbeta counter. The data is averaged to calculate an IC_{50} and % inhibition @ 1µM for the antagonist mode. For the agonist mode, an EC_{50} and percent of maximum E2 stimulation and concentration of maximum stimulation is calculated.

3-Day Rat Uterus Antagonist Assay: This model for uterine antagonism utilizes immature (3 week old) female rats that are highly sensitive to estrogenic stimulation of the uterus given that their circulating estrogen levels are prepubertal. The uteri from immature rats are fully responsive to exogenous estrogen, yet are quiescent in the absence of exogenous estrogen. Administration of exogenous estrogen to immature rats produces a reliable elevation of uterine weight, which can be used to study uterine antagonist effects. The rats are treated with both estradiol and 4 different concentrations of a compound of the present invention for 3 days and then uterine wet weights are measured.

Nineteen to twenty-one day old (or 45-50g) female rats are orally treated with E2 (0.1 mg/kg, a maximal stimulatory estrogenic stimulus for reliably increasing uterine weight) and 10, 1.0, 0.1 and 0.01mg/kg test compound for 3 days, 6 rats per group. Test compounds are dissolved in 20% β-hydroxycyclodextrin and administered by oral gavage in a volume of 0.2 mL daily (15 min. prior to the ethynyl estradiol gavage). A vehicle control, E2 alone and E2 + raloxifene are also done as controls. The animals are fasted overnight following the final dose. On the following morning, the animals are weighed, then euthanized (by carbon dioxide asphyxiation) and the uteri rapidly collected (via a mid-line ventral incision) and weighed.

Uterine weight/body weight ratios (UWR) are calculated for each animal. The percent inhibition of the estrogen-induced response is then calculated by the following formula: percent inhibition = 100 x ($UWR_{estrogen}$ - $UWR_{test\ compound}$/$UWR_{estrogen}$ - $UWR_{control}$). ED_{50} values are derived from a semi-log regression analysis of the linear aspect of the dose response curve. Both the UWR data and the percent inhibition data were statistically analyzed by one way analysis of variance (ANOVA) with post-hoc testing by Fisher's PLSD when indicated by a $p \leq 0.05$. Statistical analyses are performed using the Statview® 4.0 software package.

The compound of Example 4 was tested in the above assay and was found to inhibit the estrogen-induced response when administered at 1.0 mg/kg; ED_{50} of 0.06 mpk and a % antagonism of 89%

4-Day OVX Rat Uterine Agonist Assay: In order to assure that a test compound does not have any partial uterine agonist activity, compounds are administered to mature, ovariectomized rats.

Seventy-five day old rats are ovariectomized and treatment is started 14 days later when circulating estradiol levels have reached minimal levels. After 4 days of treatment with 3 doses of a compound of the present invention, (6 rats per group) body weight, uterine wet weight and uterine eosinophil peroxidase (EPO) activity are measured. Cholesterol levels are also measured to compare relative ability to lower cholesterol with other SERMs. If there is any question of uterine stimulation, histological examination will determine epithelial cell height.

10-Day Rat Hormone (Ovarian Stimulation) Screen: An initial, first screen for ovarian toxicity is conducted using a 10-day rat hormone study to measure estradiol and luteinizing hormone levels after compound administration. This screen is conducted by administering compound by oral gavage for 10 days to mature (9-10 week old) F344 female rats. Trunk blood is collected by rapid decapitation for evaluation of LH and estradiol levels approximately 2 hours after the 10^{th} dose. Serum, obtained by centrifugation, is removed and stored frozen below -60°C until assayed. Serum levels of LH and estradiol are measured using radioimmunoassay (RIA) methods.

Rat LH primary antibody and reference preparations (rat LH:RP-3) were obtained from Dr. A. F. Parlow, Director, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, CA. The LH assay upper limits of detection were 30 ng/mL and the lower limits of detection were 0.1 ng/mL for the 100 µl samples.

E2 Clinical Assays. DiaSorin s.r.l., Saluggia (Vercelli), Italy. The upper limit of detection was 1000 pg/mL and the lower limit of detection was 5 pg/mL. The compound of Example 4 was tested in the above assay and did not significantly elevate circulating estradiol or LH levels.

35-Day Ovary-Intact Rat Bone Assay: While previous SERMs, including raloxifene have shown efficacy in preventing bone loss in OVX rats, the possibility of interference with estrogen-regulated turnover in ovary-intact rats needs to be addressed.

This assay is done in mature rats with concentrations based on the demonstrated efficacy in the 3-day assay. Generally, at least three concentrations are chosen based on multiples of the ED_{50} generated therein. These multiples are generally 1x, 10x and 30x the ED_{50}. A compound of the present invention is administered to an OVX rat for 35 days and is compared to control, ovariectomized, and/or GnRH-administered rats. Femurs, tibiae, uteri, ovaries and serum are taken for further analyses. DEXA (Dual Energy X-ray Absorptivity), CT (Computed Tomography) and histologic analysis are done on the long bones to assess any changes. CT scans of the distal femur are done to calculate BMD (bone mineral density), cross sectional area and BMC (bone mineral content). Bone strength measurements (load to failure) may also be done to determine consequences of any bone mass or material changes. Uterine and ovarian histology are examined to confirm long term dosing effects of uterine efficacy and potential ovarian

stimulation. The serum is analyzed for LH and E2 levels as a possible indicator of ovarian effects.

Utilities

The diseases, disorders or conditions for which a compound of formula I is useful in treating include, but are not limited to, (1) uterine cancer; (2) endometriosis; (3) uterine leiomyoma/leiomyomata; (4) post-menopausal osteoporosis, *i.e.*, osteoporosis caused by the loss of bone that results from a lack of endogenous estrogen such as occurs in a woman following cessation of menstration due to natural, surgical, or other processes; and (5) estrogen receptor postive (ER+) breast cancer, particularly the prevention thereof. Treatment of uterine leiomyoma/leiomyomata as described herein, also contemplates the reduction of the occurrence or severity of the associated symptoms such as pain, urinary frequency, and uterine bleeding.

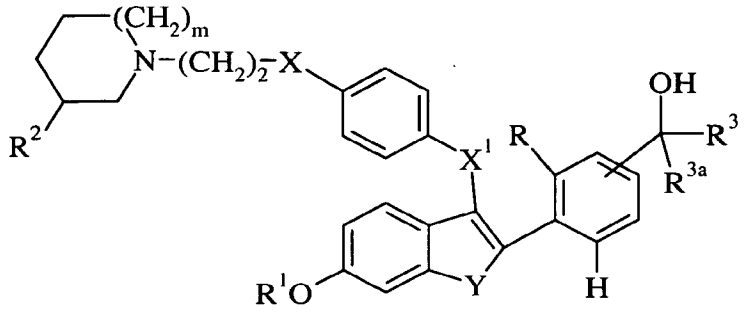
Dose

The specific dose administered is determined by the particular circumstances surrounding each situation. These circumstances include, the route of administration, the prior medical history of the recipient, the pathological condition or symptom being treated, the severity of the condition/symptom being treated, and the age of the recipient. The recipient patient's physician should determine the therapeutic dose administered in light of the relevant circumstances.

Generally, an effective minimum daily dose of a compound of formula I will exceed about 5 mg. Typically, an effective maximum daily dose will not exceed about 350 mg. The exact dose may be determined, in accordance with the standard practice in the medical arts of "dose titrating" the recipient; that is, initially administering a low dose of the compound, and gradually increasing the does until the desired therapeutic effect is observed.

WE CLAIM:

1. A compound of formula I:



I;

wherein:

m is 0, 1 or 2;

R^1 is H, SO_2(n-C_4-C_6 alkyl) or COR^4;

R^2 is H or methyl provided that if m is 1 or 2, then R^2 must be H and that if m is 0, then R^2 must be methyl;

R^3 and R^{3a} are independently H or C_1-C_6 alkyl;

X is O or NR^5;

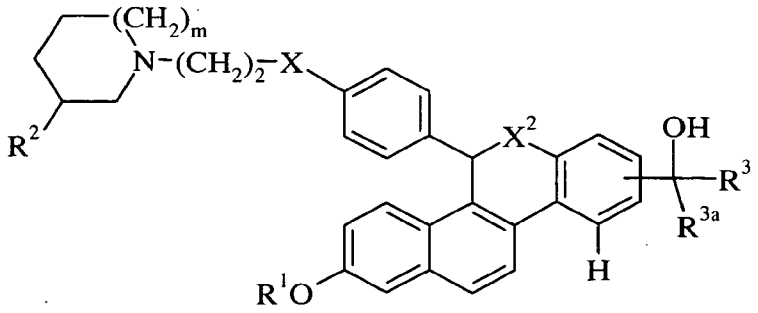
Y is S or CH=CH;

R^4 is C_1-C_6 alkyl, C_1-C_6 alkoxy, NR^6R^7, phenoxy, or phenyl optionally substituted with halo;

R^5 is H or C_1-C_6 alkyl;

R^6 and R^7 are independently H, C_1-C_6 alkyl or phenyl;

X^1 is O, CH_2 or CO and R is H or R combines with X^1 to form a moiety of the formula:



wherein X^2 is O or S;

or a pharmaceutical acid addition salt thereof.

2. The compound of claim 1 wherein R is H.

3. The compound of claim 2 wherein X and X^1 are O and m is 1 or 2.

4. The compound of claim 2 or claim 3 wherein R^1 is H or COR^4 and R^4 is C_1-C_4 alkyl, $NHCH_3$ or phenyl.

5. The compound of any one of claims 2-4 wherein R^1 is H.

6. The compound of any one of claims 2-5 wherein Y is CH=CH and m is 1.

7. The compound of any one of claims 2-6 wherein R^3 and R^{3a} are independently H or C_1-C_4 alkyl.

8. The compound of any one of claims 2-7 wherein R^3 and R^{3a} are independently H or methyl.

9. The compound of any one of claims 2-8 wherein the $COHR^3R^{3a}$ moiety is at position 4.

10. The compound of claim 1 wherein R combines with X^1.

11. The compound of claim 10 wherein X^2 and Y are O and m is 1 or 2.

12. The compound of claim 10 or claim 11 wherein R^1 is H or COR^4 and R^4 is C_1-C_4 alkyl, $NHCH_3$ or phenyl.

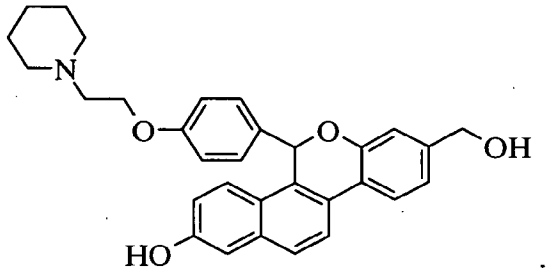
13. The compound of any one of claims 10-12 wherein R^1 is H and m is 1.

14. The compound of any one of claims 10-13 wherein R^3 and R^{3a} are independently H or C_1-C_4 alkyl.

15. The compound of any one of claims 10-14 wherein R^3 and R^{3a} are independently H or methyl.

16. The compound of any one of claims 10-15 wherein the $COHR^3R^{3a}$ moiety is at position 4.

17. A compound selected from the group consisting of:



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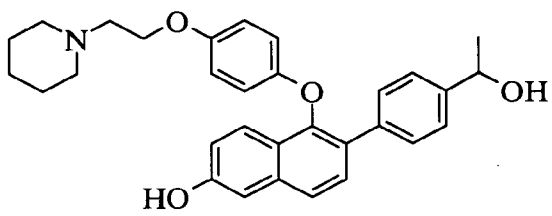

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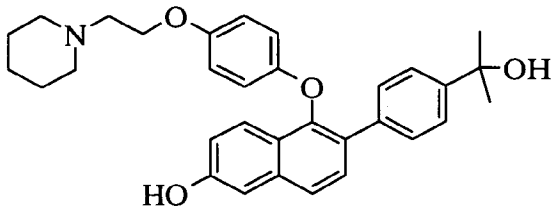


or a pharmaceutical acid addition salt thereof.

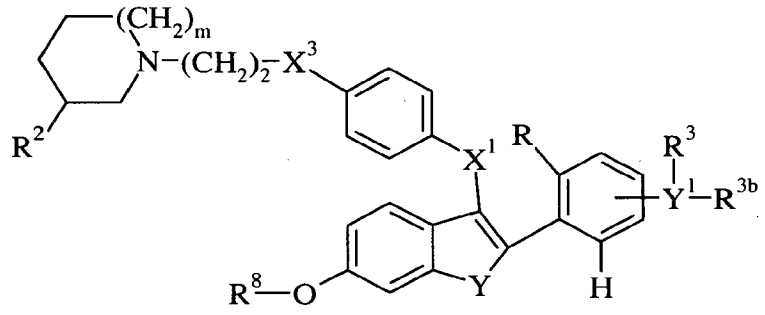
18. The compound of any one of claims 1-17 which is the hydrochloride salt.

19. A method of treating endometriosis comprising administering to a patient in need thereof an effective amount of a compound of any one of claims 1-18.

20. A method of treating uterine leiomyoma comprising administering to a patient in need thereof an effective amount of a compound of any one of claims 1-18.

21. A compound of any one of claims 1-18 for use in treating endometriosis and/or uterine leiomyoma.

22. A compound of formula II:



II;

wherein:

m is 0, 1 or 2;

R^2 is H or methyl provided that if m is 1 or 2, then R^2 must be H and that if m is 0, then R^2 must be methyl;

R^3 is H or C_1-C_6 alkyl;

R^{3b} is absent or is H or C_1-C_6 alkyl provided that if Y^1 is C(OH), then R^{3b} is H or C_1-C_6 alkyl and that if Y^1 is C=O, then R^{3b} is absent;

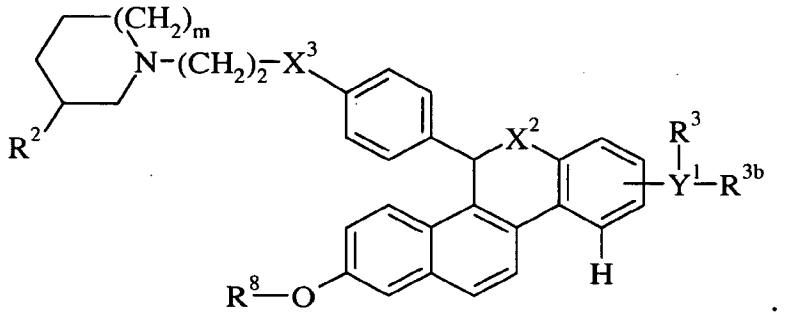
R^8 is H, C_1-C_6 alkyl, benzyl, SO_2CH_3, SO_2(n-C_4-C_6 alkyl) or COR^4;

X^3 is O or NR^9;

Y is S or CH=CH;

Y^1 is C=O or C(OH);

X^1 is O, CH_2 or CO and R is H or R combines with X^1 to form a moiety of the formula:



;

wherein X^2 is O or S;

R^4 is C_1-C_6 alkyl, C_1-C_6 alkoxy, NR^6R^7, phenoxy, or phenyl optionally substituted with halo;

R^6 and R^7 are independently H, C_1-C_6 alkyl or phenyl;

R^9 is H, C_1-C_6 alkyl or $CO_2(C_1$-C_6 alkyl); and

provided that if Y^1 is C(OH), then R^8 is C_1-C_6 alkyl, SO_2CH_3 or benzyl or X^3 is NR^9 and R^9 is $CO_2(C_1$-C_6 alkyl); or an acid addition salt thereof.

23. The compound of claim 22 wherein R is H.

24. The compound of claim 23 wherein X^3 and X^1 are O and m is 1 or 2.

25. The compound of claim 23 or claim 24 wherein R^8 is SO_2CH_3, benzyl or methyl.

26. The compound of any one of claims 23-25 wherein Y is CH=CH and m is 1.

27. The compound of any one of claims 23-26 wherein R^3 and R^{3b} are independently H or C_1-C_4 alkyl.

28. The compound of any one of claims 23-27 wherein R^3 and R^{3b} are independently H or methyl.

29. The compound of any one of claims 23-28 wherein the $Y^1R^3R^{3b}$ moiety is at position 4.

30. The compound of claim 22 wherein R combines with X^1.

31. The compound of claim 30 wherein X^3 is O and m is 1 or 2.

32. The compound of claim 30 or claim 31 wherein R^8 is SO_2CH_3, benzyl or methyl.

33. The compound of any one of claims 30-32 wherein X^2 is O and m is 1.

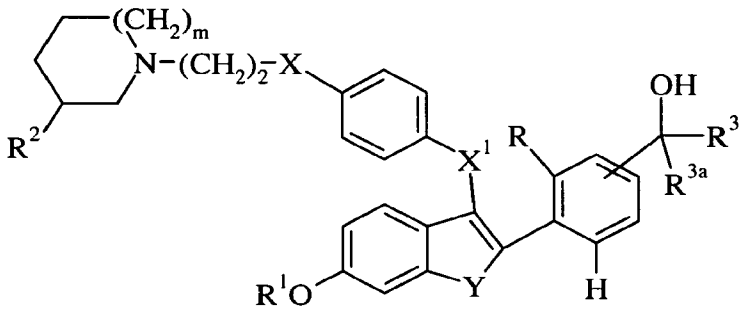
34. The compound of any one of claims 30-33 wherein R^3 and R^{3b} are independently H or C_1-C_4 alkyl.

35. The compound of any one of claims 30-34 wherein R^3 and R^{3b} are independently H or methyl.

36. The compound of any one of claims 10-15 wherein the $Y^1R^3R^{3b}$ moiety is at position 4.

ABSTRACT

The present invention relates to a selective estrogen receptor modulator of formula I:



I;

or a pharmaceutical acid addition salt thereof; useful, *e.g.*, for treating endometriosis and/or uterine leiomyoma/leiomyomata.